

CHAPTER V

CONCLUSION

Liposomes containing protein extract from *P. multocida* were prepared by double emulsion technique with various molar ratios of egg yolk lecithin to cholesterol and stabilized with various concentrations of CM-Cellulose or CM-Chitosan. The physicochemical properties such as particle size, particle size distribution, surface characteristic, releasing profile of protein as well as stability under storage at 4°C for 3 months were evaluated. The results obtained are summarized as follows.

1. Double emulsion technique was found to be a good technique for preparation of liposomes containing protein extract from *P. multocida*, by using egg yolk lecithin and cholesterol as bilayer membrane and CM-Cellulose or CM-Chitosan as stabilizers. This technique is simple, versatile and reproducible, showing high encapsulation and narrow particle size distribution. The particle size could be controlled by mechanical agitation. The median diameters were between 2.54-6.17 microns.

2. Effects of cholesterol content on the physical properties and stability.

Entrapping efficiency of protein extract from *P. multocida* increased with increasing cholesterol content. The median of particle size of freshly prepared liposomes containing protein extract from *P. multocida* was larger when the molar

ratio of egg yolk lecithin to cholesterol was increased and the entrapping efficiency was higher. The releasing profile of protein from liposomes in PBS pH 7.4 at 37°C was decreased with increasing cholesterol content. The stability in PBS pH 7.4 at 4°C for 3 months, presented by percent of remained protein was increased with increasing cholesterol content.

3. Effect of polymer coating on the physical properties and stability of liposomes.

CM-Cellulose and CM-Chitosan do not have significant differences in efficiency as liposome stabilizers. They do not disturb entrapping efficiency: The stability of liposomes coated with CM-Cellulose or CM-Chitosan increased with increasing concentration of the polymers. However, the aggregation of liposomes were found at high concentration of CM-Cellulose and CM-chitosan. The surface of polymer coated liposomes appeared as meshes around liposome vesicles.

In conclusion, the optimal proportion of egg yolk lecithin to cholesterol for preparing liposomes containing protein extract from *P. multocida* was 1:1 molar ratio. The optimal concentration of polymer was 0.02% w/v of CM-Cellulose or CM-Chitosan. The CM-Cellulose and CM-Chitosan gave the same appearance and the same properties. At high concentration the preparation appeared as a viscous liposomes resulted in obstructing of the flow of liposomes in the syringe.

In this study, the physicochemical properties of liposomes were investigated in the preparation of stable liposomes containing protein extract from *P. multocida*. However, the interaction of liposomes with cells and their immunogenicity should be further studied.